and 29-32 were rejected. Applicants have filed this divisional application canceling claims 6-12, 16-19, 23-25 and 29-32 and adding claims 33-38. Accordingly, claims 33-38 are presently being examined.

In view of the following Response, applicants respectfully request that the Examiner pass the above-identified application to issue.

Support for the Amendments

Applicants have canceled claims 6-12, 16-19, 23-25 and 29-32 and have added claims 33-38 in order to more clearly describe and distinctly claim the subject matter of applicants' method for screening candidate compounds capable of inhibiting HMGI biological activity. Specifically, applicants have added claims 33-38, as originally filed.

These amendments to the claims are fully supported in the specification as originally filed, and thus no new matter is introduced by these amendments in accord with 35 U.S.C. Section 132. Accordingly, applicants request entry of these amendments.

Remarks

Aberrations in the genetic mechanisms that control growth and proliferation have emerged as a primary event in carcinogenesis. The function of HMGI-C and HMGI(Y), two embryonically expressed DNA-binding proteins, was investigated because their expression is highly associated with tumor development. Disruptions of either HMGI-C or HMGI(Y) in humans result in a diverse array of solid mesenchymal tumors. Most prominent among these neoplasms are uterine

leiomyomata, the most common pelvic tumors in women and the indication for over 200,000 hysterectomies annually in the United States. In tumors of mammary and thyroid glands as well as in prostate cancer, HMGI expression is highly correlated with tumor progression and metastasis, suggesting that these proteins can be used for as progression markers for a variety of tumor types.

Further proof for the pivotal role of HMGI proteins in both normal and pathological growth was obtained in the mouse system. Homologous recombination was used to inactivate murine HMGI-C gene. Demonstrating the importance of the HMGI genes in growth regulation, HMGI-C knockout mice exhibit significant growth retardation (mutant mice are 60% smaller than their wild-type littermates) with the reduction in most tissues commensurate with the overall decrease in the body weight. Even more importantly, these pygmy mice are highly resistant to chemically induced skin cancer. Specifically, the frequency of tumor development in the knockout mice is 40% of that in the control animals and tumor multiplicity exhibits a 20-fold decrease. Independently, inhibition of HMGI-C synthesis was shown to render thyroid epithelial cells intransigent to retroviral transformation. At the molecular level, HMGI proteins function in transcriptional regulation by promoting cooperative binding of the transcription factors to DNA. Deregulation of the downstream target genes can easily account for the important biological roles of the HMGI proteins as well as for the dramatic consequences of their inappropriate expression.

Lipomas are one of the most common mesenchymal neoplasms in humans. They are characterized by consistent cytogenetic aberrations involving chromosome 12 in bands q14-15. Interestingly, this region is also the site of rearrangement for other mesenchymally derived tumors. The present invention demonstrates that HMGI-C, an architectural factor that functions in transcriptional

regulation, has been disrupted by rearrangement at the 12q14-15 chromosomal breakpoint in lipomas. Chimeric transcripts were isolated from two lipomas in which HMGI-C DNA-binding domains (A-T hook motifs) are fused to either a LIM or an acidic transactivation domain. These results identify the first gene rearranged in a benign neoplastic process that does not proceed to a malignancy and suggest a role for HMGI-C in adipogenesis and mesenchyme differentiation.

HMGI-C is an attractive candidate gene to be implicated in lipoma formation. This gene is required in transformation (Berlingieri et al., 1995) and is a transcriptional regulatory factor as are many genes identified at translocation breakpoints in a variety of tumors (Rabbitts, 1994). Secondly, disruption of HMGI-C leads to mice of small stature which, most intriguingly, have disproportionately less body fat than normal littermates (Benson and Chada, 1994). Finally, mouse HMGI-C maps to a region syntenic to human 12q14-15 which is the area most frequently rearranged in lipomas (Mandahl et al., 1988). Therefore, the human homolog of the mouse HMGI-C gene was cloned and its possible role in lipomas investigated.

Growth is one of the fundamental aspects in the development of an organism. Classical genetic studies have isolated four viable, spontaneous mouse mutants (Green, 1989) disrupted in growth, leading to dwarfism. Pygmy is unique among these mutants because its phenotype cannot be explained by aberrations in the growth hormone-insulin-like growth factor endocrine pathway (Lin, 1993; Li, et al., 1990; Sinha et al., 1979; Nissley et al., 1980). The present invention shows that the pygmy phenotype arises from the inactivation of HMGI-C and are critical in the assembly of stereospecific transcriptional complexes (Tjian & Maniatis, 1994). In addition, HMGI-C and the other HMGI family member, HMGI(Y)(Johnson et al., 1988), were found to be expressed predominantly during

embryogenesis. The HMGI family are known to be regulated by cell cycle dependent phosphorylation which alters their DNA binding affinity (Reeves et al., 1991). Overall, these results demonstrate the important role of HMGI proteins in mammalian growth and development.

Among the most prominent characteristics consistently exhibited by cancer cells are karyotypic aberrations which disturb genes essential for the regulation of fundamental cellular processes. A wide array of solid mesenchymal tumors is characterized by recurrent rearrangements of chromosomal bands 12q13-15 or 6p21-23. This study shows that HMGI expression is normally restricted to undifferentiated, rapidly dividing cells but is activated in differentiated adipocytes following translocations of 12q13-15 or 6p21-23 in human lipomas. The present invention shows that the molecular pathway of tumor development is dictated by the precise nature of HMGI disruption and that HMGI misexpression in a differentiated cell is a pivotal event in benign tumorigenesis.

Uterine leiomyomata are the most common pelvic tumors in women and are the indication for more than 200,000 hysterectomies annually in the United States. Rearrangement of chromosome 12 in bands q14-q15 is characteristic of uterine leiomyomata and other benign mesenchymal tumors, and a YAC spanning chromosome 12 translocation breakpoints was identified in a uterine leiomyoma, pulmonary chondroid hamartoma, and lipoma. Recently, it was demonstrated that HMGI-C, an architectural factor mapping within the YAC, is disrupted in lipomas, resulting in novel fusion transcripts. This study concerns the localization of translocation breakpoints in seven uterine leiomyomata 10 to >100 kb upstream of HMGI-C by use of fluorescence in situ hybridization. These findings suggest a different pathobiologic mechanism in uterine leiomyomata from that in lipomas. HMGI-C is the first gene identified in chromosomal rearrangements in uterine

leiomyomata and has important implications for an understanding of benign mesenchymal proliferation and differentiation.

Recently, molecular dissection of this chromosomal region has substantiated this hypothesis. To identify a gene at the breakpoint on chromosome 12 in uterine leiomyomata, a high-density physical map of the t(12;14) breakpoint region was constructed and identified a YAC, 981f11, that spans the translocation breakpoints in a uterine leiomyomata, pulmonary chondroid hamartoma and a lipoma. Further detailed characterization showed that the gene for HMGI-C, an architectural factor that is a non-histone component of chromatin, maps within 981f11 and is disrupted in lipomas. HMGI-C is rearranged in lipomas with chromosome 12 translocations, resulting in novel chimeric transcripts that fuse the DNA-binding A-T hook domains of HMGIC with potential transcriptional activation domains.

Applicants' invention, as recited in the claims, is directed to a method for screening candidate compounds capable of inhibiting HMGI biological activity which comprises the steps of:

(a) immobilizing a HMGI protein or a fragment thereof on a solid surface;

(b) incubating the HMGI protein with a candidate compound under conditions which promote optimal interaction; and

(c) measuring the binding affinity of the candidate compound to the HMGI protein or a fragment whereof; and

(d) determining from the binding affinity which candidate compounds inhibit the HMGI biological activity.

Applicants' invention is also directed to a method for screening candidate compounds capable of inhibiting HMGI biological activity which comprises the steps of:

(a) transfecting into a cell a DNA construct which contains a reporter gene under control of an HMGI protein-regulated promoter;

(b) administering to the cell a candidate compound;

(c) measuring the levels of reporter gene expression; and

(d) determining from the levels of reporter gene expression which candidate compounds inhibit the HMGI biological activity.

In view of the foregoing response, applicant requests reconsideration pursuant to 37 C.F.R. Section 112 and allowance of the claims pending in this application. Applicant requests the Examiner to telephone the undersigned attorney should the Examiner have any questions or comments which might be most expeditiously handled by a telephone conference.

Applicant's attorney authorizes the Examiner to charge Deposit Account 13-4822 if there are any additional fees due in connection with this response.

Respectfully submitted,
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